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Sterilization of *Culex pipiens fatigans* Wiedemann by Apholate

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The use of alkylating agents has been found to be a promising way of chemosterilizing mosquitos for control purposes. The investigation discussed in the present paper has shown that apholate (2,2,4,4,6,6-hexahydro-2,2,4,4,6,6-hexakis(1-aziridinyl)-1,3,5,2,4,6-triazatriphosphorine) can induce sterility rates of from 43% to 95% in Culex pipiens fatigans *Wiedemann treated in the larval stage, and from 80% to 100% in* C. fatigans *treated in the adult stage; treatment in the pupal stage produced no significant sterility.*

Of the methods of application of the apholate tested (immersion of larvae and pupae in water containing apholate, exposure of anaesthetized adults to residual deposits of apholate and dusting of anaesthetized adults with apholate powder), the dusting method was found to be effective.

Apholate induces sterility in both sexes. Females were affected much more when they were treated in the adult stage. The average number of eggs laid by a treated female was found to be reduced no matter whether the treatment was during the larval or the adult phase.

Male mosquitos chemosterilized by dusting were more competitive than normal males.

The resistance of *Culex pipiens fatigans* Wiedemann to chlorinated-hydrocarbon insecticides and organophosphorus compounds is a problem which has been assuming increasing importance over the years. In India, *C. fatigans* was the first mosquito species reported to be resistant to DDT (Pal et al., 1952). This species was also found to be resistant to dieldrin and gamma-HCH (Pal, 1958).

The resistance of various insect species of public health importance to one or more insecticides has led to the necessity of looking for other methods for their control. The eradication of the screw-worm fly from the island of Curaçao and the south-eastern parts of the USA by the release of males sterilized by gamma-radiation has greatly stimulated interest in the possibility of using certain chemicals which can produce effects similar to that of radiation (Knipling, 1959, 1960, 1962; Smith, 1963; Smith et al., 1964). LaBrecque et al. (1960) tested a number of compounds for their sterilizing effect on insects, but only a few were found to induce sterility.

The chemosterilization of mosquitos such as *Aedes aegypti*, *Ae. togoi*, *Anopheles melas*, *An. gambiae*, *An. quadrimaculatus*, *An. labranchiae*, *Culex pipiens* var. *molestus* and *C. fatigans* with different alkylating agents gave encouraging results (Weidhaas, 1962; Bertram, 1963; Weidhaas & Schmidt, 1963; Dame & Ford, 1964; Dame et al., 1964; Murrey & Bickley, 1964; D'Alessandro et al., 1966). This paper presents the results of sterilization tests carried out with the alkylating agent apholate (2,2,4,4,6,6-hexahydro-2,2,4,4,6,6-hexakis (1-aziridinyl)-1,3,5,2,4,6-triazatriphosphorine) on insectary-reared *C. fatigans*.

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MATERIAL AND METHODS

Several methods were employed for the evaluation of apholate as a sexual sterilant of *C. fatigans*. These were: (*a*) exposing the larvae or pupae to various concentrations of apholate in water, (*b*) exposing unmated adult males or females for 4, 8 or 16 hours to filter-paper treated with apholate powder, and (*c*) dusting unmated adult males and females. The different methods are described below.

Exposure of larvae to apholate in water

Larvae in their late third or early fourth instar were exposed to 40 ppm, 30 ppm, 25 ppm and 10 ppm of apholate in water. Two batches, each of

TABLE 1
EFFECTIVENESS OF APHOLATE IN WATER AS A CHEMOSTERILANT FOR *C. FATIGANS* LARVAE

Concentration (ppm)	Sex treated	No. of egg rafts [a]	Average No. of eggs per raft [a]	Percentage of unembryonated egg rafts	No. of embryonated eggs	Percentage hatched	Corrected sterility rate [b] (%)
30	Male	118 (244)	186.5	2.5	21 313	11.35	85.46
	Female	34 (130)	103.9	0.0	3 534	36.84	52.81
	Both	57 (135)	156.6	1.8	8 786	3.21	95.89
25	Male	81 (250)	181.8	5.2	14 102	10.35	86.77
	Female	52 (207)	139.7	0.0	7 265	38.06	51.25
	Both	34 (100)	136.4	2.9	4 571	4.38	94.39
20	Male	105 (280)	186.0	0.9	19 425	19.82	74.61
	Female	103 (200)	139.7	2.9	13 975	37.69	51.72
	Both	24 (62)	119.7	0.0	2 874	4.00	94.88
10	Male	65 (205)	174.1	3.1	10 926	22.21	71.55
	Female	62 (211)	140.0	0.0	8 683	44.29	43.27
	Both	72 (236)	142.6	0.0	10 268	22.59	71.06
	Neither	110 (260)	188.1	3.6	20 118	78.07	

[a] Including unembryonated egg rafts which are excluded from sterility-rate estimation. The figures in parentheses indicate the numbers of females used.
[b] Corrected by Abbott's formula.

approximately 200 larvae, were exposed to each concentration in enamel pans containing 500 ml of treated water. They were kept in the treated water up to the pupal stage. Food (yeast powder) was given to the larvae after 24 hours' exposure. Pupae were transferred to clean water and kept in cages for hatching. At 40 ppm, the emergence of adults from the pupal stage was found to be very poor. At lower concentrations, the emergence of adults was normal.

Exposure of pupae to apholate in water

Pupae of *C. fatigans* were exposed to 40 ppm and 500 ppm of apholate in water, and kept in the treated water until emergence. The adults that emerged after 24 hours' exposure were used for the experiments.

Exposure of adults to apholate on filter-paper

For these experiments, 9-cm filter-papers were smeared gently with apholate powder by means of a glass slide, and gently tapped to remove excess apholate powder. This gave an average dosage of 562 mg/ft^2.[1] About 25 unmated 2-to-3-day-old males or females were anaesthetized and placed on each treated filter-paper. The filter-papers with the mosquitos were covered with Petri dishes. In this way, mosquitos were exposed to the treated surface for 4, 8 or 16 hours. After exposure, the males and females were kept separately in cages for 24 hours before being used for different mating combinations; 16 hours' exposure produced 30% mortality, while 4 or 8 hours' exposure caused 4% mortality.

Dusting of adults

The unmated 2-to-3-day-old males and females were anaesthetized and placed separately in test-tubes containing apholate powder. The test-tubes with the apholate powder and the mosquitos were gently rolled in such a way that all the mosquitos were coated with apholate dust. Adult mortality was found to vary from 1% to 4%. After treatment, the females and males were kept separately in cages for 24 hours before being put into various combinations for mating.

In all the methods described above, the adults were segregated according to sex when less than 18 hours old. Various combinations of normal and treated females and males were then used for mass mating. After 3 to 4 days of mass mating, the blood meal was given. The egg rafts were obtained after 3 to 4 days of blood meal. Egg rafts were collected only once from each batch of females. The number of eggs in each raft was counted, and each egg raft was kept in a separate specimen tube for hatching.

[1] The dosage on the filter-papers was estimated from the difference in weight between the untreated and treated filter-papers. The average amount of apholate was found to be 38.5 mg $\pm$ 2.5 mg per filter-paper, which gives a dose of 562 mg/ft^2 $\pm$ 37 mg/ft^2 (ca 6 g/m^2).

The number of larvae hatched out of each egg raft was also counted. If few larvae, or none, hatched out of a given raft, the raft was examined to determine whether the eggs were embryonated or not. Unembryonated egg rafts were excluded from sterility-rate estimation, whereas embryonated egg rafts showing little or no larval hatching were included.

RESULTS

The results are discussed by method.

Exposure of larvae to apholate in water

The effects of various concentrations of apholate on larvae are shown in Table 1. All the concentrations tried were found to be effective in sterilizing males. Treated males mated with normal females gave 85.46%, 86.77%, 74.61% and 71.55% sterility rates at concentrations of 30 ppm, 25 ppm, 20 ppm and 10 ppm respectively. The lowest hatching rates were obtained when both sexes were treated. In such cases about 95% sterility was obtained with 30 ppm, 25 ppm and 20 ppm and about 71% with 10 ppm of apholate. Treated females mated with normal males gave the lowest sterility rates—namely, 52.81%, 51.25%, 51.72% and 43.27% with concentrations of 30 ppm, 25 ppm, 20 ppm and 10 ppm respectively.

Exposure of pupae to apholate in water

Only two concentrations (40 ppm and 500 ppm) of apholate were tried; the results obtained are shown in Table 2. At 40 ppm, no significant effect was found with either sex: about 73% to 93% hatching rates were obtained with all three combinations, as compared with about 87% in the untreated group. At 500 ppm, a slight reduction in the hatchability of eggs (to 65.0%) was found when both sexes were treated. The other two combinations gave about 77% to 84% hatching rates, as compared with about 83% in the untreated group.

TABLE 2
EFFECTIVENESS OF APHOLATE IN WATER AS A CHEMOSTERILANT FOR *C. FATIGANS* PUPAE

Concentration (ppm)	Sex treated	No. of eggs	Number hatched	Percentage hatched
40	Male	1 373	1 148	83.6
	Female	992	722	72.8
	Both	857	834	97.3
	Neither	1 242	1 086	87.5
500	Male	945	724	76.6
	Female	786	661	84.1
	Both	732	476	65.0
	Neither	4 069	3 386	83.2

Exposure of adults to apholate on filter-paper

The effect of the exposure of adults to residual deposits of apholate for 4, 8 and 16 hours are shown in Table 3. The best result was obtained when the adults were exposed to the treated papers for 16

TABLE 3
EFFECTIVENESS OF RESIDUAL DEPOSIT OF APHOLATE ON FILTER-PAPER AS A CHEMOSTERILANT FOR *C. FATIGANS* ADULTS

Exposure period (hours)	Sex treated	No. of egg rafts [a]	Average No. of eggs per raft [a]	Percentage of unembryonated egg rafts	No. of embryonated eggs	Percentage hatched	Corrected sterility rate [b] (%)
4	Male	47 (105)	141.8	0.0	6 664	11.54	87.34
	Female	35 (100)	136.6	0.0	4 780	18.05	80.20
	Both	32 (100)	115.7	3.1	3 561	3.79	95.84
	Neither	71 (120)	183.6	0.0	13 032	91.15	
8	Male	75 (170)	173.4	1.3	12 862	10.90	87.50
	Female	69 (135)	144.0	1.4	9 835	14.23	83.69
	Both	75 (175)	147.5	2.7	10 601	1.53	98.25
	Neither	107 (200)	188.8	0.9	19 998	87.23	
16	Male	74 (140)	147.9	1.3	10 801	1.05	98.79
	Female	38 (130)	118.0	0.0	4 484	7.05	91.89
	Both	18 (50)	116.9	0.0	2 104	0.0	100.00
	Neither	82 (160)	175.8	0.0	14 417	86.9	

[a] Including unembryonated egg rafts which are excluded from sterility-rate estimation. The figures in parentheses indicate the numbers of females used.
[b] Corrected by Abbott's formula.

TABLE 4
STERILITY RATES PRODUCED IN *C. FATIGANS* ADULTS DUSTED WITH APHOLATE POWDER

Sex treated	No. of egg rafts [a]	Average No. of eggs per raft [a]	Percentage of unembryonated egg rafts	No. of embryonated eggs	Percentage hatched	Corrected sterility rate [b] (%)
Male	133 (340)	148.9	2.3	19 276	4.18	94.66
Female	58 (285)	114.5	3.4	6 458	0.59	99.25
Both	70 (300)	118.6	2.9	7 978	1.35	98.27
Neither	146 (310)	167.4	1.1	24 153	78.21	

[a] Including unembryonated egg rafts which are excluded from sterility rate estimation. The figures in parentheses indicate the numbers of females used.
[b] Corrected by Abbot's formula.

hours; but this produced about 30% mortality at the end of the exposure period. Treated males mated with normal females gave 87.34%, 87.50% and 98.79% sterility after 4, 8 and 16 hours' exposure respectively. When both sexes were treated, the sterility rate was found to be 95.84%, 98.25% and 100.00% after 4, 8 and 16 hours' exposure respectively. Treated females mated with normal males gave 80.20%, 83.69% and 91.89% sterility. Thus, as with larvae, adults exposed to apholate also gave a lower sterility rate when treated females were mated with normal males.

Dusting of adults

The results obtained by this technique are shown in Table 4. All mating combinations gave about 95% or more sterility. Treated males mated with normal females gave 94.66% sterility, treated females mated with normal males gave 99.25% sterility and 98.35% sterility was obtained when both sexes were treated.

Competitiveness of sterile and normal males in mating with normal females

After 24 hours of dusting, male mosquitos were combined in various ratios with normal males to compare the vigour of treated and normal males as regards mating with normal females in 1 ft × 1 ft × 1 ft (30 cm × 30 cm × 30 cm) cloth cages. The results obtained are shown in Table 5. A 1 : 1 : 1 ratio (treated ♂♂ : normal ♂♂ : normal ♀♀) gave 77.39% sterility. This suggests that apholate dusting does not reduce the vigour of males, but actually makes them more competitive than normal males. An increase in the proportion of treated males to normal males was found to increase the sterility rate.

TABLE 5
STERILITY RATES FOR NORMAL *C. FATIGANS* FEMALES CAGED WITH VARIOUS PROPORTIONS OF NORMAL MALES AND MALES CHEMOSTERILIZED BY DUSTING WITH APHOLATE POWDER

Proportion [a]	Number of			No. of egg rafts [b]	Percentage of un-embryonated egg rafts	No. of embryonated eggs	Percentage hatched	Corrected sterility rate [c] (%)
	Treated males	Untreated males	Untreated females					
1 : 1 : 1	300	300	300	187	1.1	28 914	18.83	77.39
2 : 1 : 1	200	100	100	61	0.0	8 643	16.21	80.53
4 : 1 : 1	720	180	180	104	1.9	16 438	13.49	83.80
1 : 2 : 1	120	240	120	64	0.0	10 917	51.34	38.35
1 : 4 : 1	100	400	100	53	0.0	8 029	39.67	52.36
0 : 1 : 1	0	430	430	243	1.6	40 138	83.27	

[a] Treated males : untreated males : untreated females.
[b] Including unembryonated egg rafts, which are excluded from sterility-rate estimation.
[c] Corrected by Abbott's formula.

DISCUSSION

Apholate was found to induce sterility when the larval and adult stages were treated. Apparently, pupal stages were unaffected. The reason for this is difficult to ascertain. It might be due to the failure of apholate to penetrate the pupal skin sufficiently to produce a detectable effect on the reproductive system, to the fact that in the larval stage the apholate can enter the body through the mouth as well as through the skin, which is not possible in the pupal stage, or to the lack of any effect of apholate on the pupal stage. It would be interesting to see whether sterility could be induced in the pupal stage using a very high dose.

With larvae, all concentrations were able to induce sterility in both sexes. Murrey & Bickley (1964) induced sterility in *C. fatigans* by exposing the larvae to 10 ppm and 15 ppm of apholate. Mulla (1964) exposed larvae of *C. fatigans* continuously to 1 ppm of apholate and found only 5% hatching when both treated sexes were allowed to mate.

With adults, both methods (exposure to a residual deposit and dusting) were found to be very effective. The best results were obtained with the longest exposure (16 hours), after which 98.79% sterility was obtained when treated males were mated with normal females; but this exposure caused some mortality in the mosquitos. The dusting method was found to give better results than exposure to treated filter-paper for 4 or 8 hours: 94.66% sterility was obtained when normal females were mated with dusted males (Table 4), while the same mating combination after 4 and 8 hours' exposure produced 87.34% and 87.50% sterility respectively. The dusting method has not been reported before. The technique is very simple and no more laborious than other techniques, and allows 100-200 mosquitos to be dusted effectively in a test-tube in less than one minute's time. For more mosquitos, a bigger container can be used. Furthermore, the excess apholate can be reused after a little grinding. If the mosquitos are not over-anaesthetized the mortality will be negligible.

The average number of eggs per raft laid by treated females was found to be reduced irrespective of whether they were mated with normal or treated males (Tables 1, 2 and 4). Weidhaas (1962) using tepa and apholate and Bertram (1963) using thiotepa found a similar phenomenon with *Ae. aegypti*; here normal females mated with treated males were found to lay slightly fewer eggs.

Studies of the mating competitiveness of chemosterilized and normal males with normal females in a cage with 30-cm sides showed that a 1 : 1 : 1 ratio (chemosterilized ♂♂ : normal ♂♂ : normal ♀♀) produced 77.39% sterility. Thus, the chemosterilized males were more competitive than the normal males. However, this study should be extended using cages of different sizes to find out whether this characteristic of chemosterilized males is maintained in more spacious cages. The enhanced vigour of chemosterilized males can be considered a tremendous advantage of the mass release of sterile males for the control of insect populations, provided similar phenomena also occur with wild males.

Weidhaas & Schmidt (1963) found no loss of male vigour in *Ae. aegypti* fed on honey solution containing apholate. Murrey & Bickley (1964), who treated *C. fatigans* larvae with 10 ppm and 15 ppm of apholate, found that chemosterilized males are more competitive than normal ones. Dame & Schmidt (1964) found a reduction in the vigour of male *Ae. aegypti* and *An. quadrimaculatus* exposed to 10 mg/ft² (0.1 g/m²) of metepa on a glass surface for 4 hours. LaBrecque et al. (1962) found that male houseflies chemosterilized with apholate are more competitive than normal males.

C. fatigans males treated in the larval stage (Murrey & Bickley, 1964) or by dusting in the adult stage were found to be more competitive than normal males. Reduction of male vigour in *Ae. aegypti* and *An. quadrimaculatus* by metepa as reported by Dame & Schmidt (1964) may be due to some specific effect of the metepa.

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RÉSUMÉ

Des essais de stérilisation de *Culex pipiens fatigans* par l'apholate ont eu lieu à l'Institut national des Maladies transmissibles de Delhi, Inde.

Différentes méthodes ont été utilisées: *a*) exposition des larves et des nymphes à des concentrations variables d'apholate en solution aqueuse; *b*) exposition des adultes vierges, mâles et femelles, à l'action rémanente du produit; *c*) saupoudrage de ces mêmes adultes par le chimiostérilisant.

Appliqué au stade larvaire à la concentration de 10 à 30 parties par million, l'apholate a entraîné un taux de stérilité (exprimé par la proportion de non-éclosions parmi les œufs embryonnés) de 71 à 86% environ, après union de mâles traités et de femelles normales. Ce taux a été moindre, de l'ordre de 50%, après traitement des larves femelles uniquement. Après application du chimiostérilisant aux deux sexes, on a observé un taux de stérilité variant de 71% (pour 10 parties par million d'apholate) à 95% environ (pour des concentrations de 20, 25 et 30 parties par million). Les tentatives de stérilisation de *C. fatigans* au stade nymphal sont restées pratiquement infructueuses.

Le traitement des adultes a donné de très bons résultats. Après exposition à l'action rémanente de l'apholate pendant 16 heures, les taux de stérilité ont été respectivement de 98,79% (mâles traités–femelles non traitées), de 91,89% (mâles non traités–femelles traitées) et de 100% (traitement des deux sexes), mais la mortalité a également été élevée (30% environ). C'est cependant la méthode du saupoudrage qui s'est révélée la plus efficace, un taux de stérilité de 95% ou plus étant observé après ce traitement, indépendamment des modalités expérimentales d'accouplement.

L'étude de la concurrence sexuelle entre mâles normaux et mâles stérilisés par l'apholate a montré que chez ces derniers l'aptitude sexuelle était supérieure à celle de leurs homologues non traités.

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